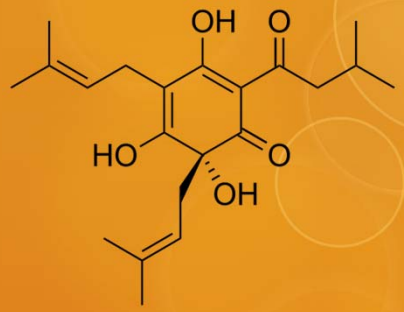
Quality Assurance Workshop

MBAA Atlanta 2012

Overview

- Bitterness Analysis
- Color Analysis
- Gram-Staining and Catalase Test
- Yeast Viability and Cell count

Bitterness in Beer



About this Analysis Method

- Method as defined in ***Methods of Analysis of the American Society of Brewing Chemists***
- Based on solvent extraction of Bittering Acids from Hops (not just iso-α-Acids)
- Does not measure perceived bitterness (Socville Units), but instead bittering chemical concentrations
- Slightly different definitions between E.B.C and A.S.B.C but both use the same scale and yield the same results.

Required Equipment and Chemicals

- Spectrophotometer (UV range)
- Shaker (optional)
- Centrifuge
- Test Tube and Stopper
- Pipettes
- 2,2,4-trimethylpentane
- 3N Hydrochloric Acid
- Beer (of course)

Methods Overview

- 10mL of Beer
- 1mL of 3N HCl
- 20mL 2,2,4-TMP
- Shake for 15 minutes
- Find absorbance @ 275nm

Color Analysis

- Uses Spectrophotometer to measure absorbance
- Based on standardized absorbance of Beer @ 430nm and 700nm
- Must be free of turbidity and degassed
- Equation => Color(SRM) = OD430*(12.5)

Catalase Test

- Biochemical test used to aid in classifying and identifying micro organisms
- Tests for the presence of catalase enzyme that is responsible for catalyzing the decomposition of hydrogen peroxide into water and oxygen
- Present in almost all organisms that are capable of thriving in oxygen environments
- **It's Cheap and Easy to perform**

Gram Staining

- "Bread and Butter" of a Microbiologist
- Should be one of the first biochemical tests performed
- Takes about 5 minutes to complete
- Gives information about the cell wall composition of an organism, specifically the presence or absence of Peptidoglycan

Staining Theory

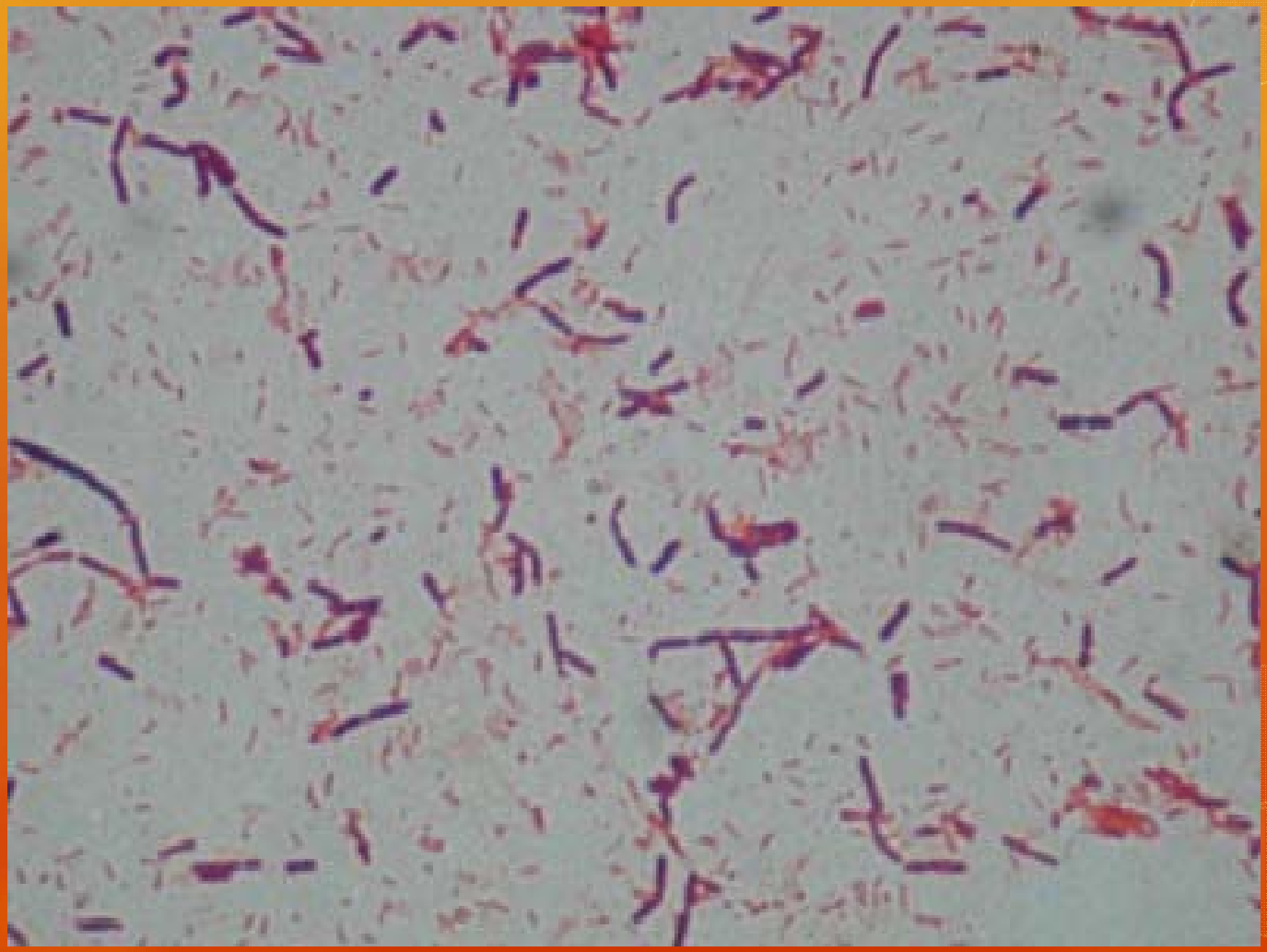
- Four part process: Stain, fix, destain, counter stain
- Crystal Violet – dark purple stain
- Gram Iodine – Mordant (fixes stain within peptidoglycan matix)
- Destain – Alcohol/ Acetone mixture that removes fixed stain from Bacteria without peptidoglycan cell wall
- Safranin – Pink/red counter stain. Dyes both Gram-negative and positive cells

Procedure

- Most procedure and staining times vary by manufacturer
- The following is for BBL Gram Stain Kit (#212539)

- Heat-fix Bacteria to microscope slide
- Crystal Violet for 1 minute
- Rinse
- Gram-Iodine for 1 minute
- Rinse
- Destain for 1-2 seconds only (Don't Over Do It)
- Rinse
- Safranin for 10-15 seconds
- Mount slide and view under microscope

Gram-Positive and Negative



Yeast Viability and Cell Count

- Uses Hemocytometer to count cells in a defined volume (1/10,000mL) and a stain to determine the percentage of viable cells
- Several stains to choose from:
  - Methylene Blue
  - Methylene Violet
  - Trypan Blue

Staining Yeast

- Sample yeast source
- Mix well (may need to add acid or EDTA to de-flocculate)
- Dilute yeast
- Remove 1mL sample and add 1mL of Methylene Blue
- Allow to stand for 15 minutes
- Mix well before addition to Hemocytometer

Counting Yeast Cells

- Count Carefully and be Consistant
- Percent Viability = ((Total cells – Stained cells)/ Total cells)*100
- Yeast Cell Count = cells counted*dilution*10,000